Possible Involvement of an Antifungal Diene in the Latency of *Colletotrichum gloeosporioides* on Unripe Avocado Fruits

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The authors acknowledge the supplying of fruits by G. A. Zentmyer and the use of equipment for ethylene treatments by I. Eaks.
This work was supported by BARD Grant I-51 80. The use of the NMR facilities, University of California, Riverside, was supported by NIH Biomedical Sciences Grant 5-S05-RR07010.
Accepted for publication 10 June 1982.

ABSTRACT

Prusky, D., Keen, N. T., Sims, J. J., and Midland, S. L. 1982. Possible involvement of an antifungal diene in the latency of *Colletotrichum gloeosporioides* on unripe avocado fruits. Phytopathology 72:1578-1582.

A preformed antifungal compound was isolated from peels of unripe avocado fruits and identified as 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene. The compound inhibited in vitro vegetative growth of *Colletotrichum gloeosporioides* and totally inhibited spore germination at 790 $\mu g \cdot ml^{-1}$. Concentrations of the diene in peels of unripe fruits were as high as 1,200 $\mu g \cdot g^{-1}$ fresh weight of peel (~1,600 $\mu g \cdot ml^{-1}$), but these decreased during ripening to about 120 $\mu g \cdot g^{-1}$ fresh weight of peel (~160 $\mu g \cdot ml^{-1}$). Concentrations of the compound decreased differentially during the ripening of two cultivars of avocado fruits that differed in rates of development of symptoms caused by *C. gloeosporioides*. The evidence supports the hypothesis that the antifungal compound is the basis for latent infections of *C. gloeosporioides* in unripe avocado peel and that subsequent active infections result from its metabolism during ripening.

Additional key words: storage, postharvest disease.

Avocado anthracnose, which is caused by *Colletotrichum gloeosporioides* Penz., is the most important fruit-rotting disease of avocado [*Persea americana* Miller var. *drymifolia* (Schlect. and Chamb.) Blake] in many countries (21). Although fruits are free of visible disease symptoms at harvest, decay lesions rapidly develop during fruit ripening and softening after 7–15 days. The large numbers of isolated lesions that develop on uninjured fruits after harvest suggested the presence of latent infections on the unripe fruits. Anatomical studies indeed revealed that appressoria persisted on unripe fruits and that further fungal development occurred only during fruit softening (1). Latent fungal infections on unripe fruits have been observed in many host-pathogen interactions (17); these may result from insufficient enzyme production to invade unripe fruits (13), nutritional requirements of the pathogen that are only supplied during fruit ripening (17), or to induced (16) and preformed (7,15,18) antifungal compounds present in unripe, but not ripening, fruits. Kashman et al (5,6) isolated a new family of natural compounds from avocado seeds and fruits; all of these were long aliphatic chains saturated at one end and highly oxygenated at the other. The compounds inhibited the growth of some bacteria (8), but neither these nor other preformed antibiotic compounds have been assessed as disease resistance factors in avocado fruits.

As part of a project to investigate the latency of *C. gloeosporioides* infections in unripe avocado fruits, we discovered a preformed antifungal compound in the peels of unripe fruits. This paper describes the identification of that compound and experiments to assess its role in anthracnose latency. Following identification of the compound, it was found to have been previously described from avocado leaves (2). Since characterization of the diene in that paper was incomplete, we report our full data here.

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0031-949X/82/12157805/$03.00/0

MATERIALS AND METHODS

Plant material and extraction procedure. Avocado fruit material was obtained from trees of the cultivars Fuerte and Hass grown in orchards at the University of California, Riverside. Fruit peel tissue 1–2 mm thick was homogenized for 1 min in 95% ethanol (150 $ml \cdot 50\ g^{-1}$ fr. wt. of tissue) with a Sorval Omnimixer operated at full speed. The filtered residue was washed twice with 50 ml of 95% ethanol, concentrated ~×3 in vacuo at 40 C, then partitioned twice with dichloromethane. The organic layers were pooled, dried with anhydrous $MgSO_4$, concentrated in vacuo, and used as a crude extract.

Crude extracts were applied to thin-layer chromatography (tlc) plates (0.375-mm silica gel GF 254, Merck), which were subsequently developed with hexanes:ethyl acetate:methanol (60:40:1, v/v). Various zones of the silica gel were scraped from the plate and extracted with ethyl acetate. Extracts were dried in vacuo, dissolved in 100% ethanol (100 $\mu l \cdot g^{-1}$ fr. wt. of tissue initially extracted), and bioassayed for antifungal activity.

Isolation and purification. The biologically active compound was isolated from the crude extract by flash chromatography on a column of silica gel H (10–40 μm, Merck) in an 8.5-cm-diameter Büchner funnel with a fritted disk. The silica gel was poured into the funnel to a depth of 6 cm, packed, and run in vacuo. The column was preequilibrated with hexanes. Considerable inactive material was eluted by hexanes followed by dichloromethane; the majority of the active compound was then eluted with 50% ethyl acetate in dichloromethane, dried in vacuo, and dissolved in 6% isopropanol in hexanes. The compound was further purified by high-performance liquid chromatography (hplc) on a Waters model 6000 A liquid chromatograph fitted with a 50 × 1.2-cm Whatman 10-μm Partisil M9 column eluted with 6% isopropanol in hexanes. The biologically active peak was rechromatographed by reversed-phase hplc on a 32 × 1.3-cm octadecylsilane-modified 5-μm Lichrosorb SI-100 column eluted with 10% water in methanol. The hplc columns were monitored with a differential refractive index detector and appropriate peaks were collected.

Identification and quantitative analysis of the antifungal compound. The highly purified compound obtained from the reversed-phase hplc column was examined by IR spectro-

photometry and NMR and mass spectrometry. Infrared spectra were acquired on a Perkin-Elmer model 202 spectrophotometer, proton NMR data on a Varian EM 390, carbon NMR data on a Bruker WH 90 Fourier transform instrument, and mass spectra on a Finnigan model 3300 system. Acetylation was done by addition of 100 μl of freshly distilled acetic anhydride and 1 ml of anhydrous pyridine to 15 mg of the purified compound and allowing the mixture to stand at room temperature overnight. The acetate derivative was recovered by evaporation of reagents and hplc (as described above) on the Partisil M9 column.

For quantitation of the antifungal diene, 50-μl aliquots of the 1.0 ml of concentrated, flash chromatographed material of the crude extract obtained from 200 g fr. wt. of avocado peels was chromatographed on the Partisil M9 hplc column as described above. Known amounts of the purified antifungal compound were used as standards in separate runs. As an internal standard, known amounts of methyl linoleate (Sigma) were added to the crude extracts before column chromatography and to the standards. Calculations of concentrations of the diene compound were based on the hplc peak heights of the compound and of the internal standard included with unknowns and standards.

Glass slide bioassay for antifungal activity. A single-spore isolate of *C. gloeosporioides* from a decayed avocado fruit was used in all experiments. The isolate was kept on potato-dextrose agar at 25 C and its pathogenicity was tested by inoculating avocado fruits once every 5 mo. Crude fruit extracts or purified compounds dissolved in 95–100% ethanol were spotted on 13-mm-diameter Millipore filters (0.45-μm pore size, Millipore Co.) that were placed in the wells of glass depression slides. After removal of the ethanol by drying, 40 μl of a suspension of spores of *C. gloeosporioides* ($\sim 10^6 \cdot ml^{-1}$) in 5% ethanol containing 0.05% polysorbate 20 (Tween-20, ICI) and 0.0015% dimethyl sulfoxide (DMSO, Mallinckrodt) were placed on the disk and the slides were incubated in a moist chamber overnight at 25 C and observed after 16 hr. In some experiments, spore suspensions were applied in 2% ethanol. Activity of the avocado compound was determined by comparing the percent germination, germ tube elongation, and appressorium formation in the treatments and in the control without crude extracts or purified avocado compound. Germ tube elongation was considered to be inhibited when length was less than half that of the control.

Biological activity in crude peel extracts. The antifungal activity of crude extracts from ripe and unripe avocado fruits (cultivar Fuerte) was determined. Ripened fruits were obtained after continuously exposing mature and immature fruits to 10 μl of ethylene per liter of air for 2 and 5 days, respectively, at 20 C. Peel of unripe fruits was extracted 1–2 hr after harvesting and that from ripe fruits was extracted when symptoms of natural infection appeared on the fruits. Peels were extracted with ethanol, and the residues were partitioned with dichloromethane as described above. The crude extracts were dissolved in 100% ethanol (76 ml/100 g fr. wt. of peel). This represented the natural concentration of the compound in the peel, since fruits of cultivar Fuerte were found to contain 76% water in the peel with no significant difference observed between ripe and unripe fruits. Extracts dissolved in methanol were spotted on Millipore filters at concentrations 0.25, 0.5, 1.0, and 1.5 times that in the peel.

Ripening of fruits and firmness determination. Harvested fruits were placed in respiratory chambers supplied with water-saturated CO_2-free air metered through glass capillary tubes. Known concentrations of ethylene were metered into the air stream through glass capillary tubes according to the method of Pratt et al (9). Concentrations of ethylene delivered to the fruit were verified by gas chromatographic measurement before the exposure period (3). All treatments were conducted in a constant-temperature room at 20 C.

Softening of the avocado tissue was determined by recording the *g* force required to cause a 5-mm probe to penetrate the flesh (skin removed) (3). The average of two penetrations on each of 10 fruits at each testing date was reported. All the experiments described above were repeated at least three times.

A. $CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-C(=O)-CH_2-CH(OH)-CH_2-O-C(=O)-CH_3$

B. $CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-C(=O)-OH$

Fig. 1. Comparison of the proposed chemical structure of **A**, the antifungal diene from avocado peel, and **B**, linoleic acid.

RESULTS

Purification and identification. The tlc plates of the crude extract from unripe cultivar Fuerte avocado fruits showed biological activity at R_f 0.57. The antifungal compound(s) could also be detected as a brown or blue spot that developed after the plates were sprayed with 50% H_2SO_4 or 3.5% phosphomolybdic acid, respectively, and heated to 110 C for 10 min. The active fraction obtained by flash chromatography was further purified by hplc on the Partisil column; it appeared after 28 min at a flow rate of 3 $ml \cdot min^{-1}$. The methyl linoleate standard appeared at a retention time of 10 min. A second purification by reversed-phase hplc was employed, since the first hplc column did not entirely separate the active compound from the tail of a UV-absorbing compound. The reversed-phase column yielded a single symmetrical peak as detected by refractive index at a retention time of 20 min at 3 $ml \cdot min^{-1}$. The purified antifungal compound showed no UV absorption in EtOH, and the IR spectrum indicated the presence of hydroxyl, carbonyl, and acetate groups: $\lambda(CHCl_3, max) = 2.9, 5.75$, and 8.0–8.3 μm, respectively. Electron impact mass spectra showed a weak molecular ion at m/e 380, and major fragment ions at 362 ($M-H_2O$), 320 (M—HOAc), 302, 243, 231, and 149. The proton NMR spectrum (90 MHz, $CdCl_3$) showed δ: 0.90 (CH_3, 3H, s), 1.32 [$(CH_2)_n$, 16H, broad s], 2.10 (CH_3COO—, 3H, s), 2.43 ($CH_2C=O$, 2H, t J = 7), 2.60 ($CH_2C=O$, 2H, d J = 6), 2.78 (bisallylic CH_2 2H, broad t), 3.15 (OH, 1H, broad s), 4.10 (AcO—CH_2—, 2H, m), 4.30 (—CHOH, 1H, broad m), 5.36 (—CH=CH—, 4H, m). These assignments were verified by proton decoupling experiments and by acetylation.

The ^{13}C spectrum in $CDCl_3$, showed (ppm): 210.8 (s, C=O), 171.0 (s, $C(=O)O$), 130.2 (d, HC=), 130.0 (d, HC=), 128.1 (d, HC=), 127.9 (d, HC=), 67.3 (t, CH_2OAc), 66.0 (d, CHOH), 45.2 (t, $CH_2-C=O$), 43.6 (t, $CH_2-C=O$), 31.5 (t), 29.6 (t), 29.3 (t), 29.3 (t), 29.1 (t), 27.2 (t, allylic CH_2), 27.2 (t, allylic CH_2), 25.6 (t, bisallylic CH_2), 23.6 (t), 22.6 (t), 20.8 (q, $CH_3C(=O)O$), 14.1 (q, CH_3). Multiplicities were determined by off-resonance decoupling at −4 ppm in the proton spectrum.

Acetylation caused a 1.1-ppm downfield shift of the 4.30 multiplet, appearance of a singlet at 2.02, and disappearance of the 3.15 δ peak. Chemical ionization mass spectra of the acetylated

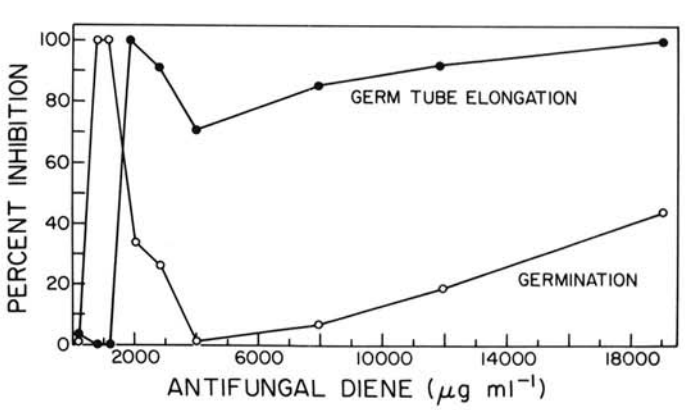

Fig. 2. Effect of the purified antifungal diene from avocado peel on conidial germination and germ tube elongation of *Colletotrichum gloeosporioides*.

compound indicated an increase of m/e 42 in the molecular weight, confirming the presence of one OH group in the native molecule. The empirical formula of the antifungal substance was assigned as $C_{23}H_{40}O_4$ from the ^{13}C spectrum and the proposed structure is Z,Z-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (Fig. 1).

Effect of the antifungal compound on *C. gloeosporioides*. The ED_{50} of the purified compound for inhibition of spore germination was about 450 $\mu g \cdot ml^{-1}$ (Fig. 2) and total inhibition was observed at 790 $\mu g \cdot ml^{-1}$. At concentrations ranging from 1,190 to 3,960 $\mu g \cdot ml^{-1}$ there was a decrease in the effect on germination, but above the latter concentration inhibition of germination again increased. At higher concentrations, inhibition of germ tube elongation was observed with 80–100% of the germinated spores.

Effect of the crude extract from avocado peel on *C. gloeosporioides*. Germination of conidia, germ tube elongation, and appressorium formation were inhibited by crude extracts from unripe cultivar Hass avocado peel (Table 1). Partial inhibition of germ tube elongation was observed when the extract from 0.25 g fr. wt. of peel was spotted on the Millipore filter. Extracts from 4 g fr. wt. of peel totally inhibited germination, and effects on germ tube elongation could not be determined. Appressorium formation was inversely related to concentration of the crude extract; appressorium formation was induced by extracts of 0.25 g, partially inhibited by extracts of 0.5 and 1.0 g, and completely inhibited with extracts of 4 g fr. wt. of unripe avocado peel.

Conidial germination was totally inhibited with crude extracts of unripe cultivar Fuerte fruit peel. However, when similar bioassay disks were transferred to PDA agar culture plates for 2–3 days the spores germinated.

When extracts were bioassayed at the relative concentrations occurring in fruit peel, extracts from unripe cultivar Fuerte fruits inhibited germ tube elongation in 78% of germinated conidia, but those from corresponding ripe fruit caused no inhibition (Table 2). Extracts from ripe fruits at ×1.5 the native concentration also did not show activity. The unripe fruit extract gave 46% inhibition when diluted to 50% the natural concentration, but no activity was observed when assayed at 25% the natural concentration.

Occurrence of the antifungal compound in avocado pears at different stages of ripening. The concentration of the antifungal compound (Fig. 3) at 1–3 days after picking varied between 700 and 1,230 $\mu g \cdot g^{-1}$ fr. wt. of peel in unripe cultivar Fuerte fruits. At 5 days after harvest, however, the concentration decreased to 350 μg and at 10 days was 125 $\mu g \cdot g^{-1}$ fr. wt., and symptoms of disease were evident. Fruit firmness decreased approximately in accordance with decrease in concentration of the antifungal diene. The biological activity of the crude extracts decreased from 55 to 6.5% inhibition of hyphal elongation during the same period, thereby indicating a very close relation between the concentration of the antifungal compound and the inhibitory activity of the crude extracts.

The relation between symptom expression of *C. gloeosporioides* and the presence of the antifungal compound in different avocado cultivars. The concentration of the antifungal compound had decreased when decay symptoms were first seen in cultivars Fuerte and Hass (Table 3). Symptoms were observed in cultivar Fuerte 6 days after harvest, and the concentration of the antifungal compound decreased from 495 to 20 $\mu g \cdot g^{-1}$ fr. wt. of peel during the period. In cultivar Hass, symptoms of decay appeared only at 11 days after harvest. Again, a decrease in concentration of the antifungal diene occurred from 385 $\mu g \cdot g^{-1}$ fr. wt. in freshly harvested fruit to 140 $\mu g \cdot g^{-1}$ fr. wt. peel at 11 days after harvest when 77% of the inoculated fruits showed decay symptoms.

Occurrence of the antifungal compound in the fruit peel of various avocado cultivars. The compound was extracted from peels of mature avocado fruits of cultivars Hass, Nabal, McArthur, and Anaheim, and these preparations yielded identical hplc retention times to those obtained from cultivar Fuerte. The NMR spectra of the extracted compounds were also identical, indicating that the same compound is present in the tested cultivars. The concentrations of the compound observed in peels of unripe fruits were Fuerte, 925; Hass, 594; Nabal, 203; McArthur, 4,412; and Anaheim, 1,616 $\mu g \cdot g^{-1}$ fr. wt. peel tissue.

TABLE 1. Effect of crude extracts from peel of unripe cultivar Hass avocado fruits on different stages of conidial development of *Colletotrichum gloeosporioides*[a]

Peel extracted (g fr. wt.)[b]	Effect of crude peel extract on: Inhibited germ tubes (% of control)[c]	Germ tubes forming appressoria[d]	Inhibition of germination (% of control)
0.25	82.2 ± 8.8	75.7 ± 6.1	0
0.50	55.6 ± 10.2	61.0 ± 4.24	0
1.00	68.3 ± 5.5	28.9 ± 4.34	15.0 ± 10.1
4.00	No germination[e]	0	100.0 ± 0

[a] The ethanol extracts were spotted on a Millipore filter and 30 μl of a suspension of conidia in 2% ethanol was added.
[b] Quantities of the antifungal diene in the crude extracts were determined by hplc as equivalent to 480 $\mu g \cdot g^{-1}$ fr. wt.
[c] Conidial germination in the 2% ethanol solution was 95 ± 4.8% with a germ tube elongation of 161.8 ± 76.5 μm after 14 hr at 25 C, compared to 58.3 ± 28.8 μm in the germ tubes inhibited by the extract from 1.0 g fr. wt. of peel.
[d] No appressoria were formed on the control conidia incubated in the absence of extracts.
[e] Could not be tested since no spores germinated.

TABLE 2. Antifungal activity present in crude extracts from peels of ripe or unripe fruits of cultivar Fuerte avocado

Fruit ripeness	Percent inhibition of germ tube elongation at extract contraction of[a]: 0.25	0.5	1.0	1.5
Ripe	0	0	0	0
Unripe	0	46.6 ± 11.0	78.1 ± 7.5	73.8 ± 2.7

[a] The relative concentration of chemicals present in fruit peel was reconstituted by dissolving the crude extracts from 100 g (fr. wt.) of peel in 76 ml of ethanol; this is referred to as concentration 1.0; other concentrations were obtained by dilution or concentration of the 1.0 extract in vacuo; data are means and standard deviations obtained from three different observations. Forty microliters of extract in ethanol were spotted onto a Millipore filter and, after drying, 40 μl of a suspension of conidia in 5% ethanol, 0.05% Tween-20, and 0.0015% DMSO were applied. Germ tube length on the water control was 150 ± 43 μm after 14 hr, compared to 49 ± 27 μm for the inhibited germ tubes at an extract concentration of 1.0.

DISCUSSION

C. gloeosporioides infects epidermal cells of unripe avocado fruits during the growing season through appressoria (1). However,

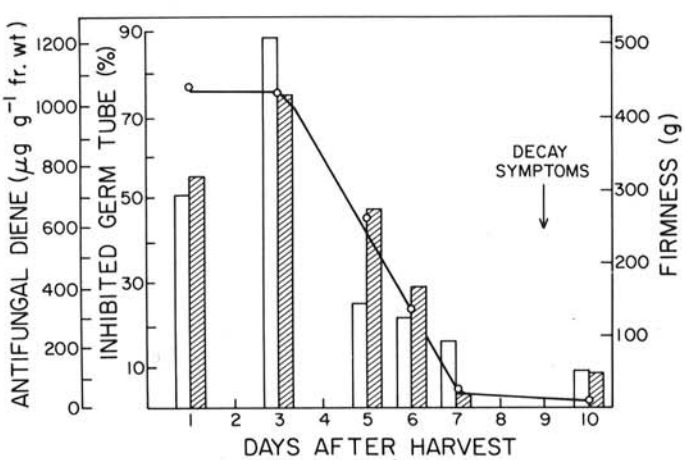

Fig. 3. Fruit firmness (o——o), antifungal activity (▨), and concentration of the antifungal diene (□) in crude extracts from peel of avocado cultivar Fuerte at different stages after harvest. The arrow denotes the first visible decay symptoms.

TABLE 3. Occurrence of the antifungal diene and symptom expression of *Colletotrichum gloeosporioides* in cultivars Fuerte and Hass avocado fruits during ethylene-induced ripening[a]

Time after harvest (days)	Fuerte		Hass	
	Antifungal diene ($\mu g \cdot g^{-1}$ fr. wt.)[b]	Appearance of symptoms[c] (% fruits)	Antifungal diene ($\mu g \cdot g^{-1}$ fr. wt)[b]	Appearance of symptoms[c] (% fruits)
0	495	0	385	0
6	20	100	210	0
11	...	...	140	77

[a] Harvested fruits were treated with 10 $\mu \cdot L^{-1}$ ethylene at 20 C for 2–3 days and then transferred to 25 C.

[b] Peel from fruits of cultivar Hass, especially when ripened, contained a compound which co-chromatographed with the methyl linoleate internal standard. Therefore, unknown samples were divided in half and the internal standard was added to only one of these. Following h.p.l.c. of both samples, the difference in height of the internal standard peak was employed to calculate the concentration of the diene compound.

[c] Darkening of the peel $\geq$5 mm in diameter was considered an infection. The fruits were inoculated by placing 20-μl drops of a spore suspension ($\sim 10^6$ spores per milliliter) at three different positions on each of 10 freshly harvested fruits.

these infections remain latent, with no further development of the fungus occurring until the fruit ripens. The presence of preformed fungitoxic compounds in unripe but not ripe fruits has previously been suggested as a possible explanation for latent fungal infections (7,15,18,19).

We isolated a single preformed antifungal compound from the peels of unripe avocado fruits and identified it as 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene. The compound had previously been described from avocado leaves (2) and had been assigned the same structure based on incomplete spectral data similar to ours. We confirmed presence of the diene in cultivar Fuerte avocado leaves and also observed it in extracts from the flesh of unripe fruit (500 $\mu g \cdot g^{-1}$ fr. wt.) and at a very low level (1.5 $\mu g \cdot g^{-1}$ fr. wt.) in the flesh of ripe fruits of cultivar Fuerte. The structurally related compounds described by Kashman et al (5,6) and Zaki et al (20) from *Persea* spp. were either not present in our extracts from fruit peel or did not exhibit antifungal activity in the bioassays.

Certain fatty acids and their derivatives have been demonstrated to be fungitoxic (4,10,11), with maximal activity at about 600 $\mu g \cdot ml^{-1}$ (12). Since linoleic acid is essentially devoid of antifungal activity, however (4,10,11), the activity of the diene is possibly due to the increased polarity resulting from the hydroxyl and acetate groups. The activity of the diene is similar to that of fatty acids up to $\sim$1,000 $\mu g \cdot ml^{-1}$, but we observed decreased activity above that concentration, possibly due to precipitation. This is similar to observations with paraffin chain salts (14). At the critical micelle concentration, aggregation of these molecules resulted in the formation of relatively small micelles that grew rapidly over a limited concentration range to a size that was constant. After attainment of that size, the addition of further solute led to a slow increase in the number of unaggregated molecules at the micellar surface. We believe that similar events probably explain the decreased toxicity of the diene from avocado at concentrations $>$1,000 $\mu g \cdot ml^{-1}$.

The antifungal diene affected various developmental stages of *C. gloeosporioides* differently. Germ tube elongation was the most sensitive stage, with higher concentrations required to prevent conidial germination. In addition, low concentrations of crude extracts enhanced appressorium formation while higher concentrations inhibited it.

The evidence presented in this paper supports, but does not prove, the hypothesis that the antifungal diene compound confers resistance to *C. gloeosporioides* in the peel of unripe avocado fruits and that its disappearance during ripening leads to renewed growth of the fungus that then may cause active, spreading lesions: (i) crude extracts from unripe fruits of cultivar Fuerte exhibited considerable antifungal activity, even after dilution to half the concentration present in the peel, but crude extracts from ripe fruits did not show any biological activity against the fungus; (ii) analyses showed that concentrations of the antifungal diene compound decreased from 1,200 $\mu g \cdot g^{-1}$ fr. wt. ($\sim$1,600 $\mu g \cdot ml^{-1}$) in unripe fruits to 120 $\mu g \cdot g^{-1}$ fr. wt. ($\sim$160 $\mu g \cdot ml^{-1}$) in ripe fruits; (iii) the in vitro activity of the purified diene against *C. gloeosporioides* (ED_{50} of 450 $\mu g \cdot ml^{-1}$ for inhibition of spore germination) is consistent with the concentrations observed in ripe and unripe fruit peels; (iv) fruits of cultivar Fuerte exhibited disease symptoms more rapidly after harvesting than cultivar Hass, and concentrations of the diene compound decreased more rapidly during ripening of the former (Table 3); and (v) most of the tested avocado cultivars possessed relatively high concentrations of the diene compound in the peel of unripe fruits, generally ranging from 500 to 4,400 $\mu g \cdot g^{-1}$ fr. wt. These factors are all consistent with the view that sufficient amounts of the antifungal diene are present in unripe, but not ripe, peel of the avocado to explain latency of the fungus in unripe fruits and renewed growth during ripening.

Attempts were made to employ unripe, but mature, avocado fruits in this work since fruit age may affect levels of the diene compound. For example, experiments with over-mature fruits of cultivar Hass during the summer months resulted in lower levels of the diene than in fruits collected during the winter. Similar effects may also have accounted for variation in the levels obtained in other cultivars, for instance, the relatively low level analyzed in cultivar Nabal.

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